

DATA EVALUATION RECORD

1. **CHEMICAL:** Metalochlor.
Shaughnessey No. 108801.
2. **TEST MATERIAL:** CGA-154281; I.D. No. FL851341; 99.3% purity.
3. **STUDY TYPE:** 72-4. Freshwater Invertebrate Flow-Through Chronic Toxicity Test. Species Tested: *Daphnia magna*.
4. **CITATION:** Hamaker, T.L. 1986. Flow-Through *Daphnia magna* Chronic Toxicity Test With CGA-154281. Study No. D801. Prepared by Environmental Research and Technology, Inc., Fort Collins, CO. Submitted by Agricultural Division, Ciba-Geigy Corporation, Greensboro, NC. EPA MRID No. 400288-29.
5. **REVIEWED BY:**

Louis M. Rifici, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: *Louis M Rifici*
Date: *3/12/93*
6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
Senior Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: *P. Kosalwat*
Date: *3/12/93*

Henry T. Craven, M.S.
Supervisor, EEB/EFED
USEPA

Signature:
Date:
7. **CONCLUSIONS:** This test is not scientifically sound. A proportional diluter was used to prepare the test solutions but the actual test concentrations were not verified analytically. Daphnid growth (length) was significantly reduced at 0.707 and 1.414 mg/l nominal concentration. Therefore, the MATC was >0.354 mg/l and <0.707 mg/l nominal concentration. The geometric mean MATC was 0.50 mg/l nominal concentration.
8. **RECOMMENDATIONS:** N/A.
9. **BACKGROUND:**
10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. MATERIALS AND METHODS:

- A. **Test Animals:** *Daphnia magna* (<24 hours old) were obtained from in-house cultures. The cultures were maintained under static conditions (20°C) and fed algae and trout chow. Adult daphnids were isolated into test dilution water prior to test initiation. Neonates used in testing were obtained from these adults.

- B. **Test System:** A closed-cell pressure operated intermittent-flow proportional diluter was used. The test solutions were allowed to flow through the test system for 24 hours prior to test initiation. Flow into the test chambers resulted in greater than five volume replacements. A flow splitting box was used to distribute the test solutions to the four replicate test chambers per concentration.

The test chambers were 1-l glass beakers with 297- μ m screen collars. As fresh solution entered the beakers, the test solution overflowed and was collected in the surrounding aquaria for disposal.

The dilution water was reconstituted water prepared from dechlorinated tap water. The water was adjusted to a hardness of 100 mg/l as CaCO₃ with the addition of inorganic salts and aerated before use in the test.

The test stock solution was prepared by dissolving the test material in methanol.

- C. **Dosage:** Twenty-one-day flow-through test. Based on the 48-hour EC₅₀ (3.5 mg/l, nominal) from a definitive static acute toxicity test, five nominal concentrations (0.088, 0.177, 0.354, 0.707, and 1.414 mg/l), a solvent control, and a dilution water control were used. The solvent control and highest test concentration solutions had a methanol concentration of 100 mg/l.

- D. **Design:** Ten daphnids were randomly distributed to each replicate. The daphnids were fed *Selenastrum capricornutum* through a food reservoir.

On days 0, 3, 6, and 8, live and dead daphnids were recorded and dead organisms were removed. Beginning on day 10 and continuing approximately every other day until the end of the test, live and dead adult daphnids were counted and neonates were counted and removed. Chambers were cleaned with a stiff brush and laboratory

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detergent once per week. At test termination, surviving adults from each chamber were measured to the nearest 0.1 mm.

Test solution temperature was recorded daily. The dissolved oxygen concentration (DO) was measured twice weekly. Hardness, alkalinity, conductivity, and pH were measured weekly.

- E. **Statistics:** The survival of dilution water control and solvent control daphnids was compared using a 2X2 contingency table and Chi-square analysis. Survival in the exposures was compared to the pooled control results using a contingency table and Scheffe's multiple comparison test. Daphnid length was subjected to the $F_{\max}$ test to determine homogeneity of variance within each treatment level and control group. Significant differences between the length of the pooled control and the treatment daphnids were determined using one-way analysis of variance (ANOVA) and Dunnett's test. Reproduction in the dilution water control and solvent control was compared using one-way ANOVA. The number of young produced per adult per reproductive day in the exposures was compared to the solvent control using ANOVA. The level of significance in all tests was $p \leq 0.05$.
12. **REPORTED RESULTS:** On day 6, replicate A of the 0.707 mg/l treatment was accidentally discarded. At test termination, survival of daphnids exposed to 1.414 mg/l was significantly reduced compared to survival of daphnids in the pooled controls (Table 3-2, attached). Survival in the remaining treatments was $\geq 76.7\%$ and statistically comparable to the pooled control.

The release of the first brood occurred on day 10 and day 13 for the solvent control and dilution water control groups, respectively. For the treatment groups, the release of the first brood occurred on days 11 through 13. The average number of young produced in the dilution water control and solvent control was 42.6 and 35.8 young, respectively. "Methanol may have depressed the reproductive rate somewhat during this test." Reproduction in the treatment groups was not significantly different from solvent control reproduction (Table 3-3, attached).

Mean length for the pooled control and exposure groups was listed in Table C-3 (attached). Compared to the pooled

controls, daphnid length was significantly reduced in the two highest test concentrations after 21 days.

During the test, the DO was 5.1-6.8 mg/l. The pH values were 7.8-7.9 and the conductivity was 319-320 " μ mhos/cm²." The temperature was 19.0-20.8°C. Hardness and alkalinity were 100-102 and 74.5-77.0 mg/l as CaCO₃, respectively.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

The no-observed-effect concentration (NOEC) and lowest-observed-effect concentration (LOEC) were 0.354 and 0.707 mg/l nominal concentrations, respectively. The geometric mean maximum acceptable toxicant concentration (MATC) was 0.50 mg/l nominal concentration.

The laboratories Quality Assurance officer stated that "The flow-through *Daphnia magna* chronic toxicity test with CGA 154281 was conducted in accordance with Good Laboratory Practice Guidelines."

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

- A. Test Procedure: This study is not scientifically sound. Significant deviations from the guidelines are as follows:

A proportional diluter was used to prepare the test solutions but the actual test concentrations were not verified analytically.

Forty percent mortality occurred in the solvent control replicate B. More than 30% mortality in the control is considered unacceptable.

The dilution water was reconstituted water prepared from dechlorinated water. Residual chlorine in the dilution water should have been measured and reported.

No transitional period between light and dark photoperiod was used in the test.

A description of the test material (i.e., physical state, color) was not included in the report.

- B. Statistical Analysis: Daphnid survival data (arcsine square root transformed), the number of young produced per adult per reproductive day, and daphnid length were analyzed using one-way ANOVA and Bonferroni's test (Toxstat Version 3.3). Survival at 1.414 mg/l was

significantly lower than dilution water control survival (printout 1). Dilution water control reproduction and solvent control reproduction were significantly different. Reproduction at 1.414 mg/l was significantly lower than dilution water control reproduction (printout 2). The raw length data were submitted but illegible, therefore only mean length could be statistically analyzed. The results were the same as the author's (printout 3).

- C. Discussion/Results: The solvent control and highest test concentration contained the highest concentration of methanol used in the test (100 mg methanol/l). Using a density of 0.79 g/ml for methanol (Handbook of Chemistry and Physics, 55th edition), the maximum solvent concentration was 0.13 ml/l. Visual inspection of the means for daphnid survival and reproduction revealed that responses in the four lowest test concentrations were more like those of the dilution water control, than the solvent control. Since these four lowest concentrations contained much less solvent than that in the solvent control, the reviewer used the responses of the dilution water control daphnids for statistical comparisons. It appears that the concentration of the solvent may have negatively affected the reproduction and survival of the daphnids since these parameters in the solvent control and highest test concentration were always lower than the dilution water control. It is possible that only the length data show any significant effect based on toxicant exposure.

The reports states that, during the test, the test containers were cleaned with a stiff brush with laboratory detergent and rinsed. It is unclear whether the rinsing was adequate to remove detergent residues from the containers.

This test is not scientifically sound. A proportional diluter was used to prepare the test solutions but the actual test concentrations were not verified analytically. Daphnid growth (length) was significantly reduced at 0.707 and 1.414 mg/l nominal concentration. Therefore, the MATC was >0.354 mg/l and <0.707 mg/l nominal concentration. The geometric mean MATC was 0.50 mg/l nominal concentration.

- D. Adequacy of the Study:

(1) Classification: Invalid.

(2) **Rationale:** A proportional diluter was used to prepare the test solutions but the actual test concentrations were not verified analytically.

(3) **Repairability:** No.

15. COMPLETION OF ONE-LINER FOR STUDY: Yes, 03-04-93.

400288-29, metalochlor, daphnid survival
 File: a:400288.29 Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro Wilks test for normality
 Data PASS normality test at P=0.01 level. Continue analysis.

Bartlett's test for homogeneity of variance
 Data PASS homogeneity test at 0.01 level. Continue analysis.

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	0.392	0.065	3.160
Within (Error)	20	0.413	0.021	
Total	26	0.805		

Critical F value = 2.60 (0.05,6,20)

Since F > Critical F REJECT Ho: All groups equal

BONFERRONI T-TEST - TABLE 1 OF 2 Ho: Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	dilution contrl	1.254	0.900		
2	solvent contrl	1.123	0.800	1.294	
3	0.088	1.178	0.850	0.750	
4	0.177	1.149	0.825	1.035	
5	0.354	1.219	0.875	0.349	
6	0.707	1.068	0.767	1.693	
7	1.414	0.867	0.575	3.811	*

Bonferroni T table value = 2.61 (1 Tailed Value, P=0.05, df=20,6)

BONFERRONI T-TEST - TABLE 2 OF 2 Ho: Control < Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	dilution contrl	4			
2	solvent contrl	4	0.205	22.8	0.100
3	0.088	4	0.205	22.8	0.050
4	0.177	4	0.205	22.8	0.075
5	0.354	4	0.205	22.8	0.025
6	0.707	3	0.225	25.0	0.133
7	1.414	4	0.205	22.8	0.325

400288-29, metalochlor, daphnid reproduction
 File: a:40028829.dt2 Transform: NO TRANSFORMATION

Shapiro Wilks test for normality
 Data PASS normality test at P=0.01 level. Continue analysis.

Bartlett's test for homogeneity of variance
 Data PASS homogeneity test at 0.01 level. Continue analysis.

t-test of Solvent and Blank Controls		Ho:GRP1 MEAN = GRP2 MEAN	
GRP1 (SOLVENT CTRL) MEAN =	5.1815	CALCULATED t VALUE =	4.6162
GRP2 (BLANK CTRL) MEAN =	3.5213	DEGREES OF FREEDOM =	6
DIFFERENCE IN MEANS =	1.6602		

TABLE t VALUE (0.05 (2), 6) = 2.447** SIGNIFICANT DIFFERENCE at alpha=0.05
 TABLE t VALUE (0.01 (2), 6) = 3.707** SIGNIFICANT DIFFERENCE at alpha=0.01

ANOVA TABLE				
SOURCE	DF	SS	MS	F
Between	6	9.958	1.660	3.027
Within (Error)	20	10.966	0.548	
Total	26	20.924		

Critical F value = 2.60 (0.05,6,20)
 Since F > Critical F REJECT Ho:All groups equal

BONFERRONI T-TEST		TABLE 1 OF 2		Ho:Control<Treatment	
GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	dilution contrl	5.181	5.181		
2	solvent contrl	3.521	3.521	3.171	*
3	0.088	4.924	4.924	0.491	
4	0.177	4.747	4.747	0.830	
5	0.354	5.063	5.063	0.227	
6	0.707	4.325	4.325	1.514	
7	1.414	3.801	3.801	2.636	*

Bonferroni T table value = 2.61 (1 Tailed Value, P=0.05, df=20,6)

BONFERRONI T-TEST		TABLE 2 OF 2		Ho:Control<Treatment	
GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	dilution contrl	4			
2	solvent contrl	4	1.368	26.4	1.660
3	0.088	4	1.368	26.4	0.257
4	0.177	4	1.368	26.4	0.434
5	0.354	4	1.368	26.4	0.119
6	0.707	3	1.478	28.5	0.856
7	1.414	4	1.368	26.4	1.380

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400288-29, metalochlor, mean daphnid length
 File: a:40028829.dt3 Transform: NO TRANSFORMATION

Shapiro Wilks test for normality
 Data PASS normality test at P=0.01 level. Continue analysis.

Bartlett's test for homogeneity of variance
 Data PASS homogeneity test at 0.01 level. Continue analysis.

t-test of Solvent and Blank Controls Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CTRL) MEAN =	4.4153	CALCULATED t VALUE =	0.3402
GRP2 (BLANK CTRL) MEAN =	4.3929	DEGREES OF FREEDOM =	6
DIFFERENCE IN MEANS =	0.0224		

TABLE t VALUE (0.05 (2), 6) = 2.447 NO significant difference at alpha=0.05
 TABLE t VALUE (0.01 (2), 6) = 3.707 NO significant difference at alpha=0.01

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	0.472	0.079	4.673
Within (Error)	20	0.337	0.017	
Total	26	0.809		

Critical F value = 2.60 (0.05,6,20)

Since F > Critical F REJECT Ho:All groups equal

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	dilution contrl	4.415	4.415		
2	solvent contrl	4.393	4.393	0.244	
3	0.088	4.255	4.255	1.745	
4	0.177	4.312	4.312	1.125	
5	0.354	4.358	4.358	0.625	
6	0.707	4.091	4.091	3.277	*
7	1.414	4.051	4.051	3.973	*

Bonferroni T table value = 2.61 (1 Tailed Value, P=0.05, df=20,6)

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	dilution contrl	4			
2	solvent contrl	4	0.240	5.4	0.022
3	0.088	4	0.240	5.4	0.160
4	0.177	4	0.240	5.4	0.103
5	0.354	4	0.240	5.4	0.057
6	0.707	3	0.259	5.9	0.325
7	1.414	4	0.240	5.4	0.365

Page ____ is not included in this copy.

Pages 10 through 14 are not included in this copy.

The material not included contains the following type of information:

- ☐ Identity of product inert ingredients.
- ☐ Identity of product impurities.
- ☐ Description of the product manufacturing process.
- ☐ Description of quality control procedures.
- ☐ Identity of the source of product ingredients.
- ☐ Sales or other commercial/financial information.
- ☐ A draft product label.
- ☐ The product confidential statement of formula.
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Ecological Effects Branch One-Linear Data Entry Form

Chemical Metolachlor

Shaughnessy No. 48108801

Pesticide Use

INVERTEBRATE ACUTE TOXICITY	% AI	EC ₅₀ (95%CL) SLOPE	HRS/ TYPE	NOEC	STUDY/REVIEW DATES	MRID/ CATEGORY	LAB	RC
1.								
2.								
3.								
4.								
5.								
6.								
7.								
CHRONIC TOX.	% AI	MATC LC ₅₀	DAYS	AFFECTED PARA.	STUDY/REVIEW DATES	MRID/ CATEGORY	LAB	RC
1. <u>Daphnia magna</u>	99.3	>0.354 mg/l ⁺ <0.707 mg/l NA	21	length, survival, repro.	1986/1993	400288-29 1994/1d	ERT	LMP
2.								
3.								

COMMENTS: * nominal concentration

TITLE: 400288-29, metalochlor, daphnid survival

FILE: a:400288.29

TRANSFORM: ARC SINE(SQUARE ROOT(Y))

NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	dilution contrl	1	1.0000	1.4120
1	dilution contrl	2	0.8000	1.1071
1	dilution contrl	3	0.9000	1.2490
1	dilution contrl	4	0.9000	1.2490
2	solvent contrl	1	0.8000	1.1071
2	solvent contrl	2	0.6000	0.8861
2	solvent contrl	3	0.9000	1.2490
2	solvent contrl	4	0.9000	1.2490
3	0.088	1	0.9000	1.2490
3	0.088	2	0.8000	1.1071
3	0.088	3	0.9000	1.2490
3	0.088	4	0.8000	1.1071
4	0.177	1	0.9000	1.2490
4	0.177	2	0.9000	1.2490
4	0.177	3	0.7000	0.9912
4	0.177	4	0.8000	1.1071
5	0.354	1	0.8000	1.1071
5	0.354	2	1.0000	1.4120
5	0.354	3	0.8000	1.1071
5	0.354	4	0.9000	1.2490
6	0.707	1	0.8000	1.1071
6	0.707	2	0.7000	0.9912
6	0.707	3	0.8000	1.1071
7	1.414	1	0.4000	0.6847
7	1.414	2	0.4000	0.6847
7	1.414	3	0.8000	1.1071
7	1.414	4	0.7000	0.9912

TITLE: 400288-29, metalochlor, daphnid reproduction

FILE: a:40028829.dt2

TRANSFORM: NO TRANSFORMATION

NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	dilution contrl	1	5.5750	5.5750
1	dilution contrl	2	4.6620	4.6620
1	dilution contrl	3	5.5470	5.5470
1	dilution contrl	4	4.9420	4.9420
2	solvent contrl	1	3.4640	3.4640
2	solvent contrl	2	4.3170	4.3170
2	solvent contrl	3	3.2690	3.2690
2	solvent contrl	4	3.0350	3.0350
3	0.088	1	3.8840	3.8840
3	0.088	2	4.9220	4.9220
3	0.088	3	5.7530	5.7530
3	0.088	4	5.1380	5.1380
4	0.177	1	5.4540	5.4540
4	0.177	2	4.6300	4.6300
4	0.177	3	3.3970	3.3970
4	0.177	4	5.5070	5.5070
5	0.354	1	5.3670	5.3670
5	0.354	2	5.6390	5.6390
5	0.354	3	4.1340	4.1340
5	0.354	4	5.1110	5.1110
6	0.707	1	3.1330	3.1330
6	0.707	2	5.6350	5.6350
6	0.707	3	4.2080	4.2080
7	1.414	1	3.4880	3.4880
7	1.414	2	3.8260	3.8260
7	1.414	3	3.7160	3.7160
7	1.414	4	4.1750	4.1750

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TITLE: 400288-29, metalochlor, mean daphnid length

FILE: a:40028829.dt3

TRANSFORM: NO TRANSFORMATION

NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	dilution contrl	1	4.4925	4.4925
1	dilution contrl	2	4.3688	4.3688
1	dilution contrl	3	4.4667	4.4667
1	dilution contrl	4	4.3333	4.3333
2	solvent contrl	1	4.5000	4.5000
2	solvent contrl	2	4.4550	4.4550
2	solvent contrl	3	4.3583	4.3583
2	solvent contrl	4	4.2583	4.2583
3	0.088	1	4.0750	4.0750
3	0.088	2	4.3594	4.3594
3	0.088	3	4.2833	4.2833
3	0.088	4	4.3031	4.3031
4	0.177	1	4.3000	4.3000
4	0.177	2	4.2833	4.2833
4	0.177	3	4.2964	4.2964
4	0.177	4	4.3688	4.3688
5	0.354	1	4.3725	4.3725
5	0.354	2	4.3500	4.3500
5	0.354	3	4.2844	4.2844
5	0.354	4	4.4250	4.4250
6	0.707	1	3.9844	3.9844
6	0.707	2	4.2000	4.2000
6	0.707	3	4.0875	4.0875
7	1.414	1	4.2300	4.2300
7	1.414	2	3.6750	3.6750
7	1.414	3	4.0875	4.0875
7	1.414	4	4.2107	4.2107